CONCISE REPORT

Glucose-6-Phosphate Dehydrogenase of Malaria Parasite
Plasmodium falciparum

By Akira Yoshida and Eugene F. Roth, Jr

Plasmodium falciparum growth is impaired in glucose-6-phosphate dehydrogenase (G6PD)-deficient red blood cells (RBCs), and malaria has been implicated in the spreading of deficient variants in malaria-endemic areas. Recent reports suggest that the malaria parasite can adapt itself to grow in these variant RBCs by producing its own G6PD, but studies on parasite G6PD are very limited. In this report, we define the properties of the parasite G6PD. G6PD was partially purified from infected and uninfected variant RBCs associated with severe G6PD deficiency. G6PD from infected RBCs contained two components separable by starch gel electrophoresis: a major component (~90% activity) with a very slow anodal electrophoretic mobility and a minor component (~10% activity) with the same mobility as the host G6PD. Parasite G6PD exhibited much higher affinity (low K_m) to G6P and nicotinamide-adenine dinucleotide phosphate (NADP) than did human G6PD. Southern blot hybridization indicated that the parasite genome contained nucleotide sequences that were hybridizable with the human G6PD cDNA. These data indicate that the parasite is capable of adapting to G6PD-deficient RBCs by producing its own G6PD.

G E N E T I C D E F I C I E N C Y of the X chromosome-linked glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: nicotinamide-adenine dinucleotide phosphate [NADP] oxidoreductase, EC 1.1.1.49; G6PD) is common among the populations of present and past malaria-endemic areas. For example, Gd A(-), associated with 10-15% of normal red cell G6PD activity, is found in 10% to 20% of black populations; and Gd Mediterranean B(-), associated with a more severe deficiency, is commonly found among Mediterranean, Southeast Asian, and South Pacific populations. It is generally accepted that malaria has been a major selective pressure for the spread of these common deficiency variants in these populations. In vivo and in vitro growth of Plasmodium falciparum has been found to be retarded in G6PD-deficient red blood cells. In red blood cells, formation of reduced glutathione (GSH) depends solely on nicotinamide-adenine dinucleotide phosphate (NADPH) generated by the hexose monophosphate shunt pathway. A lower GSH level in G6PD-deficient red cells drastically impairs adenosine triphosphate (ATP)-phosphoribosyl-transferase (EC 2.4.2.17) activity, presumably causing insufficient production of 5'-phosphoribosyl-1-pyrophosphate to permit parasite growth.

Early studies on animal malaria parasites (P lophurae, P berghei, and P knowlesi) indicated that the parasites demonstrated Embden-Meyerhof pathway activity, but no measurable activity of the hexose monophosphate shunt pathway. Recent reports, however, have suggested that P falciparum contains its own G6PD, and can adapt itself to grow in G6PD-deficient red blood cells. This paper reports the nature of G6PD produced by P falciparum and the existence of nucleotide sequences in the parasite genome that are hybridizable with the human G6PD cDNA.

MATERIALS AND METHODS

P falciparum (FCR-3 strain), grown in normal human red blood cells (obtained from Drs. S. Schulman and J. Vanderberg, New York University School of Medicine), was cultured in an in vitro system described by Jensen and Trager. G6PD-deficient blood was obtained from a Persian-Jewish male donor described in the previous studies. The parasites were continuously grown in the G6PD-deficient red cells, and were enriched for trophozoite/schizont stages by gelatin sedimentation, which generally provided parasitemias of from 40% to 85%. The parasite-enriched cells were washed with isotonic phosphate-buffered saline, containing 10 mmol/L glucose, and kept frozen at −70°C.

For partial purification of G6PD, the red blood cells (infected and uninfected host cells and control normal cells) were hemolyzed with 2 vol of 5 mmol/L phosphate, pH 6.4, containing 0.1 mmol/L NADP, and 0.1 volume of toluene. The stroma-free hemolysate was treated with a diethyl aminoethyl (DEAE)-cellulose (DE-22 Whatman, Kent, England) column, as previously described, to eliminate hemoglobin and other contaminants. The enzyme was precipitated with ammonium sulfate (40 g/100 mL at pH 6.4); redissolved in an adequate amount of 0.01 mol/L phosphate, pH 7.0, containing 10 μmol/L NADP and 1 mmol/L 2-mercaptoethanol; and dialyzed against the same buffer. The G6PD activity was spectrophotometrically assayed.

Starch gel electrophoresis was performed, as previously described, and the enzyme was visualized by G6PD-activity staining.

For the study of the parasite genome, DNA was prepared from the infected host red blood cells (about 10 mL) following the previous method with some modifications. Briefly, DNA was extracted from the cells with 9 vol of 0.1 mol/L Tris-HCl, pH 7.4, containing 12.5 mmol/L EDTA, 1% sodium dodecylsulfate, and proteinase K (500 μg/mL), at 37°C for two hours. DNA was purified by stepwise treatments with chloroform-phenol, chloroform-isamylalcohol, phenol, and ether, and precipitated with ethanol. Approximately 800 μg of parasite DNA was obtained. The human DNA was prepared from leukocytes by the previous method.
The genomic DNA samples were digested by restriction endonucleases (EcoR I, Hind III, Pst I, or Msp I), and the digests were separated by electrophoresis in 0.8% agarose gel and transferred onto a nitrocellulose filter. The filter was hybridized with the 32P-labeled cDNA probes, as previously described.

RESULTS AND DISCUSSION

The anodal electrophoretic mobility of the parasite G6PD was much slower than either the host human G6PD or the control normal G6PD B(+) at pH 8.6 and at pH 7.0 (Fig 1). Judging from the relative intensity of activity staining, more than 90% of the activity can be attributed to the parasite G6PD and the minor remainder to the host G6PD in the partially purified enzyme prepared from the parasite-infected host red cells.

The G6PD activity of the parasite-infected host red cells was 0.23 units/g hemoglobin, whereas that of the uninfected host red cells was 0.01 units and that of the control normal red cells was 6.2 units. The kinetic properties (Km values for G6P and NADP, and the rate of utilization of substrate analogues) are shown in Table I. Parasite G6PD exhibits very low Km for both G6P and NADP; therefore, parasite G6PD is more active than human G6PD in red blood cells, where G6P and, particularly, NADP concentrations are lower than the Km values of the human G6PD. The pH optima of parasite G6PD was found to be 7.5 to 9.0, which is similar to that of the normal G6PD B(+).

The electrophoretic mobility and kinetic properties of the host G6PD are the same as those reported for G6PD Mediterranean B(−), i.e., the male donor is a Gd B(−) hemizygote.

Southern blot hybridization analysis indicated that the parasite genome contained nucleotide sequences that were homologous to the human G6PD cDNA (Fig 2). The parasite’s DNA was found to be hybridizable also with the human alcohol dehydrogenase cDNA, aldehyde dehydrogenase cDNA, and aldolase A cDNA, but not with the human phosphoglycerate kinase (PGK) (X-linked) cDNA (Southern blot hybridization patterns are not shown). Since PGK is one of the most evolutionally conservative proteins and a key enzyme for ATP generation in the glycolytic pathway, the lack of nucleotide sequences hybridizable with the PGK cDNA in the parasite’s genome was rather unexpected.

Hemplemann and Wilson reported that the putative P. falciparum G6PD migrated as a slow-moving band, while, according to Usanga and Luzzatto, the parasite G6PD had the same electrophoretic mobility as normal human G6PD B(+). The enzymatic properties of the putative parasite G6PD were not reported. The present study clearly indicates that the parasite G6PD has very slow anodal electrophoretic mobility and exhibits unique kinetic properties. The discrep-

Table 1. Properties of P. falciparum G6PD

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Electrophoretic Mobility</th>
<th>Km (μmol/L)</th>
<th>Analogue Utilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 8.6</td>
<td>pH 7.0</td>
<td>G6P</td>
</tr>
<tr>
<td>Normal B (+)</td>
<td>100</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>Mediterranean B (−)</td>
<td>100</td>
<td>100</td>
<td>19-26</td>
</tr>
<tr>
<td>Host G6PD</td>
<td>100</td>
<td>100</td>
<td>27</td>
</tr>
<tr>
<td>Malaria G6PD</td>
<td>63</td>
<td>55</td>
<td>11</td>
</tr>
</tbody>
</table>

Enzyme activity was assayed in a reaction mixture containing 0.1 mol/L Tris-HCl, pH 8.0, 10 mmol/L MgCl2, 0.6 mmol/L G6P (or 2-deoxy G6P), and 0.2 mmol/L NADP (or deamino NADP) at 25°C. The values of analogue utilization are the ratio (%) of enzyme activities assayed in the presence of natural substrates (i.e., G6P and NADP) and in the presence of the substrate analogues (i.e., 2-deoxy G6P and deamino NADP). Km values were determined using the reaction mixtures containing various concentrations of G6P of NADP. Electrophoretic mobility is expressed taking that of normal G6PD B(+) as 100. Values of Mediterranean G6PD B(−) are based on previous data. Contamination of host G6PD in the malaria G6PD preparation is less than 10% (Fig 1).
ancy could be due to the difference in the malaria parasite strains used.

In conclusion, *P. falciparum* can produce its own G6PD in G6PD-deficient host red blood cells. The parasite-G6PD activity of the infected deficient cells (measured in vitro under optimal assay conditions) reaches about 5% of the activity of normal red cells. Parasite G6PD has a high affinity to the substrates, and could be physiologically more active than human G6PD. The parasite may sustain its own growth and is capable of adapting to G6PD-deficient red blood cells by producing its own G6PD.

ACKNOWLEDGMENT

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REFERENCES

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